only bring stability to the fibers, but also determine the specific orientation of individual peptides within these nanofibers.

2241-Pos Board B11
Second Virial Coefficients as Determined using Self Interaction Chromatography and Protein Aggregation in Solution
Amanda Quigley, Daryl Williams.
Imperial College, London, United Kingdom.
One of the key parameters for describing protein self-interactions in solution is the second virial coefficient; B_{22}. Measurement of the second virial coefficient is important for describing the solution behaviour of proteins, especially as it relates to precipitation, aggregation and crystallisation phenomena. Due to its quick analysis time and use of small amounts of protein, Self Interaction Chromatography (SIC) is becoming an increasing popular approach. This paper includes a detailed description of the best experimental practise for running SIC experiments on aqueous protein solutions.
SIC data is presented here for a series of protein systems studied including lysozyme and lactoferrin, for a wide range of salts and their concentrations. The aggregation kinetics for these two proteins have also been obtained using Dynamic Light Scattering (DLS) for the same solution conditions. Remarkably, SIC confirmed the repulsive nature of B_{22} values of lactoferrin for all salts and concentrations tested. This data agreed completely with DLS data which confirmed the exception stability of lactoferrin to aggregation in solution. In contrast, aggregation behaviour of lysozyme was a strong function of salt species present as well as their concentrations. This aggregation behaviour correlated well with B_{22} values obtained by SIC. This work confirming the value of SIC as a useful technique for predicting the aggregation behavior of proteins in solution via B_{22} data.

2242-Pos Board B12
The Role of Interfaces in the Nucleation of Amyloid Nanotubes
Neil R. Anthony, David G. Lynn, Keith M. Berland.
Emory University, Atlanta, GA, USA.
The nucleation and growth pathways of cross-ß peptide aggregates are widely studied but not fully understood. Using Amyloid-ß(16-22), the nucleating core of the Amyloid-ß protein, and a rhodamine 110 -labeled peptide (Rh1722) that co-assembles with Amyloid- ß(16-22) nanotubes without changing morphology, we investigate the role of interfaces in amyloid nucleation and self assembly. The high sensitivity of the Rhodamine 110 lifetime to its local environment provides a metric for structural heterogeneity, which is exploited in these studies using Fluorescence Lifetime Imaging Microscopy (FLIM). Specifically, we identify the importance of air-solvent and solvent-glass interfaces in the nucleation of cross-ß peptide nanostructures and apply FLIM to demonstrate that different interfaces can lead to distinct nucleation and self-assembly pathways and also to differences in the structure of assembled peptides.

2243-Pos Board B13
Single Molecule Studies of Interaction Between Alzheimer's Amyloid-β Peptides of different Lengths
Pavithra Aravamudhan, Ari Gafni, Duncan Steel.
University of Michigan, Ann Arbor, MI, USA.
The oligomers formed by amyloid-β (Aβ) peptides are thought to play a causative role in Alzheimer's disease, possibly due to membrane permeabilization by small Aβ oligomers. Two primary variants of Aβ, of lengths 40 (Aβ40) and 42 (Aβ42) amino acids are produced at defined ratios in a normal individual. Changes in the ratio of Aβ40/Aβ42 have been shown to correlate strongly with the disease. Further, a mixture of the two forms of the peptide has been shown to exhibit different fibrillization kinetics in vitro and to elicit different extents of cellular toxicity. Thus, mixed Aβ40/Aβ42 oligomers can have different characteristics compared to oligomers formed by either one of the peptides. However, most research in the field focuses on one of the peptides at a time. Further, the Aβ oligomers are heterogenous, metastable and physiologically occur at low nanomolar concentrations, which makes it difficult for the use of conventional techniques to identify the toxic oligomers. In our work, we use single molecule methods to overcome these obstacles and study the formation and the evolution kinetics of mixed oligomers of Aβ40 and Aβ42 at different ratios in solution. We employ single molecule FRET and photobleaching of two different fluorophores attached to the N-terminal of Aβ40 and Aβ42 to determine the oligomer size and composition. Extending these studies to oligomer formation and binding kinetics of the two peptides on model membranes constructed from brain lipid extracts will provide a critical new understanding of how the stoichiometry of interaction of the two peptides both in solution and on the membrane surface affects the composition and permeabilizing potency of the resulting mixed oligomers.

2244-Pos Board B14
Structures and Dynamics in Amyloid-Beta Dimers: Effects of Zinc Binding and Chelation
Robin K. Lammi, Emily Amenson, Rebecca Mitchum, Abigail Bradner, Iris Strzyzewski.
Winthrop University, Rock Hill, SC, USA.
Alzheimer's disease (AD) is causally linked to the self-association of amyloid-β peptide (Aβ), a small protein of 39-43 amino acids. Historically, Aβ fibrils found in extracellular senile plaques were thought to be the pathogenic agents; however, recent evidence suggests that Aβ oligomers as small as dimers are more closely linked to Alzheimer's symptoms and progression. We have employed Förster resonance energy transfer (FRET) measurements to probe structures and dynamics in dimers of Aβ40. Results for single, surface-tethered dimers reveal two characteristic FRET efficiencies, perhaps indicative of two preferred dimer structures; these values are confirmed by fluorescence lifetime measurements in bulk solution. Here, we report comparative studies in the absence and presence of zinc, which is thought to promote Aβ oligomer formation at synapses. The effects of the zinc chelator clioquinol in reversing zinc-induced structural change will also be discussed.

2245-Pos Board B15
Comparison of Epitaxially and Solution-Grown Amyloid ß25-35 Fibrils
Ünige Murvai[1], Judit Somkuti[1], Katalin Soós[2], Botond Penke[3], László Smeller[1], Miklós S.Z. Kellermayer[1].
[1]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, [2]Department of Medical Chemistry, University of Szeged, Szeged, Hungary, [3]Supramolecular and Nanostructured Materials Research Group of the Hungarian Academy of Sciences, Szeged, Hungary.
Amyloid fibrils play a key role in a wide range of protein misfolding disorders. Amyloid ß peptides form self-associating fibrillar structures in Alzheimer's disease. The biologically active, toxic fragment of the full-length beta peptide, the Aß25-35 peptide is able to form an oriented network on mica by an epitaxial assembly mechanism. Whether the structure of the epitaxially grown fibrils is similar or identical to that of the fibrils assembled under equilibrium conditions is not known. To explore these differences, we investigated fibril structure and dynamics with atomic force microscopy, force spectroscopy (AFM) and Fourier transform infrared (FTIR) spectroscopy methods.
According to our AFM experiments the epitaxially-grown fibrils were significantly different from solution-grown fibrils in terms of their morphology and formation kinetics. The fibril height was 1-3 nm for the epitaxially-grown fibrils, whereas solution-grown fibrils were 7-40 nm thick. Unlike epitaxialy-grown fibrils, fibrils assembled in solution displayed a presumably helical structure. While fibril assembly in solution occurred on a time scale of hours to days, on mica surface fibrils appeared within a few minutes. The nanomechanical behavior of Aß25-35 fibrils was characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of protofilaments. Both types of fibrils showed similar plateau-like nanomechanical responses, however the plateau-force distribution was unimodal for epitaxially-grown fibrils and bimodal for solution-grown fibrils. The IR spectra contained an intense peak indicative of beta-sheet structure: 1630 cm-1 and 1623 cm-1 for epitaxially grown fibrils and for fibrils assembled in solution respectively. The shift in the amide I band towards smaller wavenumbers indicates a more compact structure. Thus, while both fibrils types display an underlying beta-sheet structure, they are slightly different: solution-grown fibrils are more compact with a pronounced axial periodicity.

2246-Pos Board B16
Cellular Internalization of Monomeric and Oligomeric Amyloid-Beta 42 Peptide
Sha Jin[1,2], Jan Bieschke[2].
[1]Humboldt-Universität zu Berlin, Berlin, Germany, [2]Max Delbrueck Center for Molecular Medicine, Berlin, Germany.
Amyloid beta (Abeta) is the major component of extracellular plaques found in Alzheimer's disease (AD). Uptake of Abeta from extracellular to intracellular space, which is likely by endocytosis, appears to be an important process for understanding AD pathology (Friedrich et al., PNAS 2010; Hu et al., PNAS 2009). It may also be a promising target for treatment and prevention.
We aimed to study biophysical details of Abeta uptake, and to determine the uptake kinetics for different Abeta forms: monomers, oligomers, higher molecular mass aggregates of Abeta in and small fibrils. We used neuroblastoma (Sh-EP) cells as a model for neurons. Uptakes kinetic were followed by using the fluorescent labelled Abeta 42 that was monomerized and was purified by gel filtration. Abeta 42 peptide was added to the cell culture medium and the amount of intracellular aggregates was quantified using automated fluorescence microscopy.

We compared uptake kinetics and aggregation kinetics in buffer and in cell culture medium at different Abeta 42 concentrations to test whether aggregation precedes uptake or vice versa. To compare the uptake of different Abeta species, pre-aggregated Abeta oligomers or small fibrils were added to the cells. We found that pre-aggregation accelerated the formation of intracellular aggregates, which suggests that Abeta oligomers and / or small fibrils may be taken up more rapidly than Abeta monomer.
In the future the form and location of intracellular Abeta will be monitored by high resolution fluorescence nanoscopy combined with atomic force microscopy.

2247-Pos Board B17
Biophysical Studies on Protein Aggregation and Amyloid Fibril Formation
Mily Bhattacharya, Neha Jain, Priyanka Dogra, Soumyadyuti Samai, Smita Patil, Samrat Mukhopadhyay.
Indian Institute of Science Education and Research (IISER), Mohali, Mohali, India.
Protein misfolding leading to aggregation and amyloid fibril formation has been implicated in a number of debilitating human disorders. The primary causative agent is commonly identified to be an aberrant misfolded-form of a protein that self-assembles into oligomers which eventually lead to the formation of ordered cross-β-rich amyloid fibrils. The transiently-populated oligomeric intermediates enroute to amyloid assembly have drawn considerable attention owing to their higher cytotoxicity compared to that of mature amyloid fibrils. Our efforts are directed towards unraveling the mechanisms of amyloid fibrillation using a diverse array of biophysical tools involving steady-state and time-resolved fluorescence, circular dichroism, Raman spectroscopy, dynamic light scattering, electron microscopy and atomic force microscopy [1-3]. Our recent findings on aggregation of an all α-helical protein namely serum albumin revealed that low pH-induced partially-unfolded, molten-globule-like conformers associate to form obligatory oligomeric intermediates that serve as precursors to amyloid fibrils. Comparison of the kinetics of protein conformational- and size changes using multiple structural probes in-tandem indicated that oligomerization followed by conformational conversion leads to the formation of β-rich fibrils. Recently, we have extended our biophysical studies to other proteins such as ovalbumin. I will also discuss our recent results on chain collapse and oligomerization of intrinsically disordered proteins that are capable of forming amyloid fibrils.
References:
1. M. Bhattacharya, N. Jain and S. Mukhopadhyay. ''Insights into the Mechanism of Aggregation and Fibril Formation from Bovine Serum Albumin'', ***J. Phys. Chem. B*** **2011,** *115,* 4195-4205.
2. M. Bhattacharya, N. Jain, K. Bhasne, V. Kumari and S. Mukhopadhyay. ''pH-induced conformational isomerization of bovine serum albumin studied by extrinsic and intrinsic protein fluorescence'', ***J. Fluoresc.*** **2011,** *21,* 1083-1090.
3. N. Jain,* M. Bhattacharya* and S. Mukhopadhyay. ''Chain collapse of an amyloidogenic intrinsically disordered protein'', ***Biophys. J.*** **2011** (In press).
**Equal contribution.*

2248-Pos Board B18
Single Molecule Fluorescence Studies of Amyloid-Beta 1-42 Aggregation
Jennie A. Flint, Priyanka Narayan, Mathew H. Horrocks, Sarah L. Shammas, David Klenerman.
University of Cambridge, Cambridge, United Kingdom.
The proteolytic cleavage of the transmembrane amyloid precursor protein (APP) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, $A\beta_{1-40}$ and $A\beta_{1-42}$, are the major components of the extracellular amyloid plaques characteristic of Alzheimer's disease (AD). Within these plaques, the Aβ is found aggregated into long polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity (1). Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous (2). Equimolar mixtures of $A\beta_{1-42}$ singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using single molecule fluorescence confocal microscopy and FRET, allowing the characterisation of oligomers present during aggregation of monomers and disaggregation of fibrils. Additionally, we have extended our single-molecule studies to examine the species formed during the co-aggregation of $A\beta_{1-40}$ and $A\beta_{1-42}$ to understand the interaction at physiological concentrations and ratios. The thorough detection and characterisation of these potentially toxic oligomeric species provides a basis with which to screen therapeutic agents and other modulators of aggregation *in vitro* which could inform *in vivo* studies in the future.
1. F. Chiti, C.M. Dobson, *Annu. Rev. Biochem.* 75, 333-366 (**2006**)
2. A. Orte *et al*., *PNAS*, 105, 14424-14429 (**2008**)

2249-Pos Board B19
Replica Exchange Statistical Temperature Molecular Dynamics Simulations of Peptide Dimerization
Alan van Giessen, Matthew Church, Christine Ferry.
Hobart and William Smith Colleges, Geneva, NY, USA.
We present a new computer simulation algorithm called Replica Exchange Statistical Temperature Molecular Dynamics that combines the multicanonical sampling technique of Statistical Temperature Molecular Dynamics with temperature replica exchange. This algorithm is related to Wang-Landau sampling but uses a dynamical update of the density of states to achieve flat energy sampling within a replica-dependent temperature range. This algorithm is used to investigate the thermodynamics of dimerization of two polypeptide chains. Three two-peptide systems are investigated: two hydrophobic peptides, two hydrophilic peptides, and one of each. Each monomer is modeled using a coarse-grained peptide model that has an α-helix as the lowest energy configuration. For each dimer system, interesting folding behavior is observed. It is found that at low temperatures, both peptides are helical and the lowest-energy configuration maximizes inter-peptide contact; at high temperatures, both peptides are random coils; and at intermediate temperatures, one peptide is folded and the other unfolded. Formation of the peptide dimer causes one peptide to fold at a *higher* temperature than an isolated monomer and the other to fold at a *lower* temperature than an isolated monomer. Dimerization causes one peptide to become more stable and the other peptide to become less stable. It is also shown that at intermediate temperatures, neither peptide adopts a random coil configuration: the helical peptide induces a conformational change in the unfolded peptide. The Potential Energy Surface (PES) is also determined for each dimer and the effect of hydrophobic/hydrophilic nature of the peptide on the PES is discussed.

2250-Pos Board B20
Protein Structure in Amorphous Solid Phase
Sheila Khodadadi, Susan Krueger, Joseph E. Curtis.
NIST, Gaithersburg, MD, USA.
Protein-based medicines often require freezing or stabilization in carbohydrate glasses for storage prior to use. The structural stability of these proteins is of the great importance in the conditions required for pharmaceutical purposes. Problems involving aggregation and stability of the protein in freeze-dried formulations are of the challenges for the pharmaceutical industry.
Small-angle neutron scattering (SANS) is uniquely qualified to study the structure of proteins in the liquid and solid phases that are biotechnologically relevant for proteins. The structural and conformational changes of a model protein, lysozyme, during the destabilizations in water- ice and carbohydrates systems were studied using SANS and MD simulations. X-ray diffraction measurements were performed to verify existence of cubic and hexagonal ice structures in protein-ice system. Measurements and modelling efforts to understand protein structural changes will be discussed and the interaction distances measured by SANS and proposed model protein structures in different carbohydrate glasses will be compared.

2251-Pos Board B21
Osmolyte Effect on Aggregation of ß-Lactoglobulin Amyloid-Prone Peptides by Explicit Molecular Dynamics Simulation
Stéphane Abel[1], **Nicolas Taulier**[2], Wladimir Urbach[3], Marcel Waks[2].
[1]CEA, Saclay, France, [2]CNRS, Paris, France, [3]ENS, Paris, France.
Whereas the toxicity of pathogenic amyloids relies on protein misfolding, other nonpathogenic or even functional amyloid structures can regulate physiological activity in a number of domains. Understanding the structural transition within this class of amyloids will provide insights into the general mechanism for ordered to agregation-dependent transitions. We have performed explicit molecular dynamics simulations using GROMACS with GROMOS53A6 force field and SPC water model. We have investigated 6 and 12 peptides of sequence: Ac-y^{146}HIRLSFN^{152}NH_2, from bovine ß-lactoglobulin; theses peptides are known to display high aggregation propensity under specific experimental conditions (5 M urea). We have shown by MD that the peptides form in water and in 5 M urea, in less than 100 ns, a structural aggregate displaying antiparallel ß-sheets, with an hydrophobic core protected from water. Furthermore, we have examined the effect of two different osmolytes (2.5 and 5 M Urea and 1.5 M Trehalose) on the nature of the interactions favoring the ß-structure of